

CHAGAS' DISEASE: SELECTIVE AFFINITY AND
CYTOTOXICITY OF *TRYPANOSOMA CRUZI*-IMMUNE
LYMPHOCYTES TO PARASYMPATHETIC GANGLION CELLS

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The megaesophagus and megacolon endemic in South America are related to Chagas' disease. These mega conditions are found in patients with chronic Chagas' infection, when the parasite is not demonstrable in the lesions. These are characterized by depopulation of parasympathetic ganglion cells, dilation and hypertrophy of the viscera. In the experiments described here we demonstrate a selective affinity and adherence of Trypanosoma cruzi-immune lymphocytes to myenteric, parasympathetic ganglion cells, leading to neuronolysis. None of these features are observed when non-immune lymphocytes from control rabbits are used, or when the immune lymphocytes are allowed to react with CNS neurons. This demonstration is an indication of the high degree of specificity of the destruction of parasympathetic neurons in Chagas' disease. We postulate that the T. cruzi-immune lymphocyte rejection of parasympathetic neurons, but not of CNS neurons, might be related to recognition of a cross-reacting antigenic determinant secreted only by the target neurons. In favor of this interpretation is the observation of lymphocytic infiltrates and parasympathetic ganglion cell destruction in chronic Chagas' infection in the absence of encephalitis.

Chagas' disease can be manifested clinically by the involvement of segments of the digestive tract (Ramos & Oria, 1940; Köberle & Nador, 1955). Usually the esophagus and/or the terminal portion of the colon are the segments involved. These "mega" conditions are described as dilation of the viscera, which show marked hypertrophy of their walls. This lesion has been related to depopulation of the myenteric, parasympathetic ganglion cells (Köberle & Nador, 1955). The pathogenesis of ganglion cell destruction in

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Chagas' disease remains to be elucidated. In this study we tested some hypotheses that have been raised as attempts to explaining the pathogenesis of endemic, South American megaesophagus and megacolon related to Chagas' disease: 1) The direct action by the intracellular parasites; 2) The secretion of neurotoxin by the *Trypanosoma cruzi*, the causative agent of the disease; 3) The action of antibody and other humoral factors; 4) cytotoxicity of *T. cruzi*-immune lymphocytes to the parasympathetic ganglion cells.

This paper describes a selective affinity and cytotoxicity of *T. cruzi*-immune lymphocytes to parasympathetic ganglion cells.

MATERIAL AND METHODS

Strains of T. cruzi: Two strains of *T. cruzi* were used in these experiments. The Ernestina strain was isolated from a child with acute Chagas' disease in São Felipe – Bahia in 1964 (Neva & Gam, 1977). The Albuquerque strain of *T. cruzi* was isolated from a farmer who acquired the infection in the county of Barreiras, on the west bank of the São Francisco river in the state of Bahia – Brazil. The patient displayed a chagoma and developed a severe infection that required medical care. The trypomastigote forms of *T. cruzi* in the peripheral blood reached 4×10^4 parasites/ml. These trypomastigotes were transferred from the patients blood to tissue culture. Both strains of *T. cruzi* are maintained in VERO-cell cultures in our laboratory.

Inoculation of rabbits with trypomastigote forms of the Ernestina strain of T. cruzi: Six, one to two month-old, New Zealand white rabbits received a single inoculation of 19^6 trypomastigotes / Kg via IP. All the animals showed positive parasitemia in the acute phase of the infection, which was demonstrated by xenodiagnosis.

Collection of sera and demonstration of humoral antibodies to T. cruzi: Sera were collected from normal, non-Chagasic rabbits and from rabbits 1 to 6 inoculated with *T. cruzi* one to two years previously. Blood was collected from a marginal ear vein and allowed to clot. The humoral antibodies to *T. cruzi* were demonstrated by the direct agglutination of epimastigote forms of the parasite (Vattuone & Yanovsky, 1971) and by the indirect hemagglutination test, as described in a previous paper (Teixeira & Santos-Buch, 1974).

Isolation of blood mononuclear cells and assays of inhibition of mononuclear cell migration: Blood mononuclear cells were isolated from 40 ml of heparinized venous blood collected from normal rabbits and from rabbits with chronic Chagas' disease. The ficoll-hypaque gradient centrifugation method of Boyum (1968) was used for separation of the cells. The capillary tube method of migration of the blood mononuclear cells was used, as described in a previous paper (Teixeira & Santos-Buch, 1974). The cells were allowed to migrate in the presence and in the absence of antigens of *T. cruzi*. In other experiments lymphocytes were isolated from monocytes by previous incubation of these cells to allow adherence of the monocytes on plastic surface. The lymphocytes in the supernatant were used.

Preparation of T. cruzi antigens: Trypomastigote and amastigote forms of the Ernestina and of the Albuquerque strains of *T. cruzi* were each harvested repeatedly from VERO-cell cultures. The cell debris were removed by centrifugation at $200g \times 5$ min at $4^\circ C$ and the supernatant containing the parasite forms were disrupted in a high rotation tissue homogeniser (Teixeira & Santos-Buch, 1974). The total homogenates (TH) were partitioned by differential centrifugation to yield the microsomal antigens ($30.000g \times 35$ min fraction) and the soluble antigens, the supernatant of the $100.000g \times 90$ min fraction, or cytosol. A spinco model ultracentrifuge and a 50 Ti rotor were used.

Cultivation of fetal rabbit central nervous system tissue (CNS) and inoculation of nervous cells with T. cruzi: A twenty-one day pregnant rabbit underwent an abdominal incision under pentobarbital anesthesia and the fetuses were secured under aseptic technique. The fetal skull was opened and the brain was transferred into PBS, pH 7.2. The cortical tissue was minced and the cells were released by trypsinisation. A final concentration of 10^6 viable cells/ml suspended in 10 ml of MEM with 5% rabbit serum were used to seed the 75 ml plastic culture flasks (Falcon Plastics, Oxnard, California). The culture medium was supplied with 100 IU/ml of penicillin and 100 μ g/ml of streptomycin.

After five days of cultivation at 37°C the neurones and the glial cells formed a monolayer. The cultures were then inoculated with 5×10^5 trypomastigotes, either of the Ernestina or of the Albuquerque strain of *T. cruzi*. Seven to ten days after inoculation the cultures were heavily parasitized.

Isolation and cultivation of myenteric parasympathetic ganglion cells: Four-to-six month-old, male, New Zealand white rabbits were treated as for a surgical procedure. The chow pellets were withdrawn and the rabbits were fed an excess of neomycin and aureomycin by the oral route. After 48-72 h the animal underwent a medial abdominal incision under anesthesia and the proximal portion of the ileum was cut. A 5 ml sterile pipette was introduced into the ileum. Blood flow was maintained by vessels in its distal portion. A strip of the external muscle layer, containing viable parasympathetic ganglia was delaminated and wrapped, spiral-shaped, on a glass rod (Fig. 1). This preparation was immediately transferred into 15 ml snap-capped tubes (Falcon Plastics) containing MEM with 5% rabbit serum, and supplied with antibiotics.

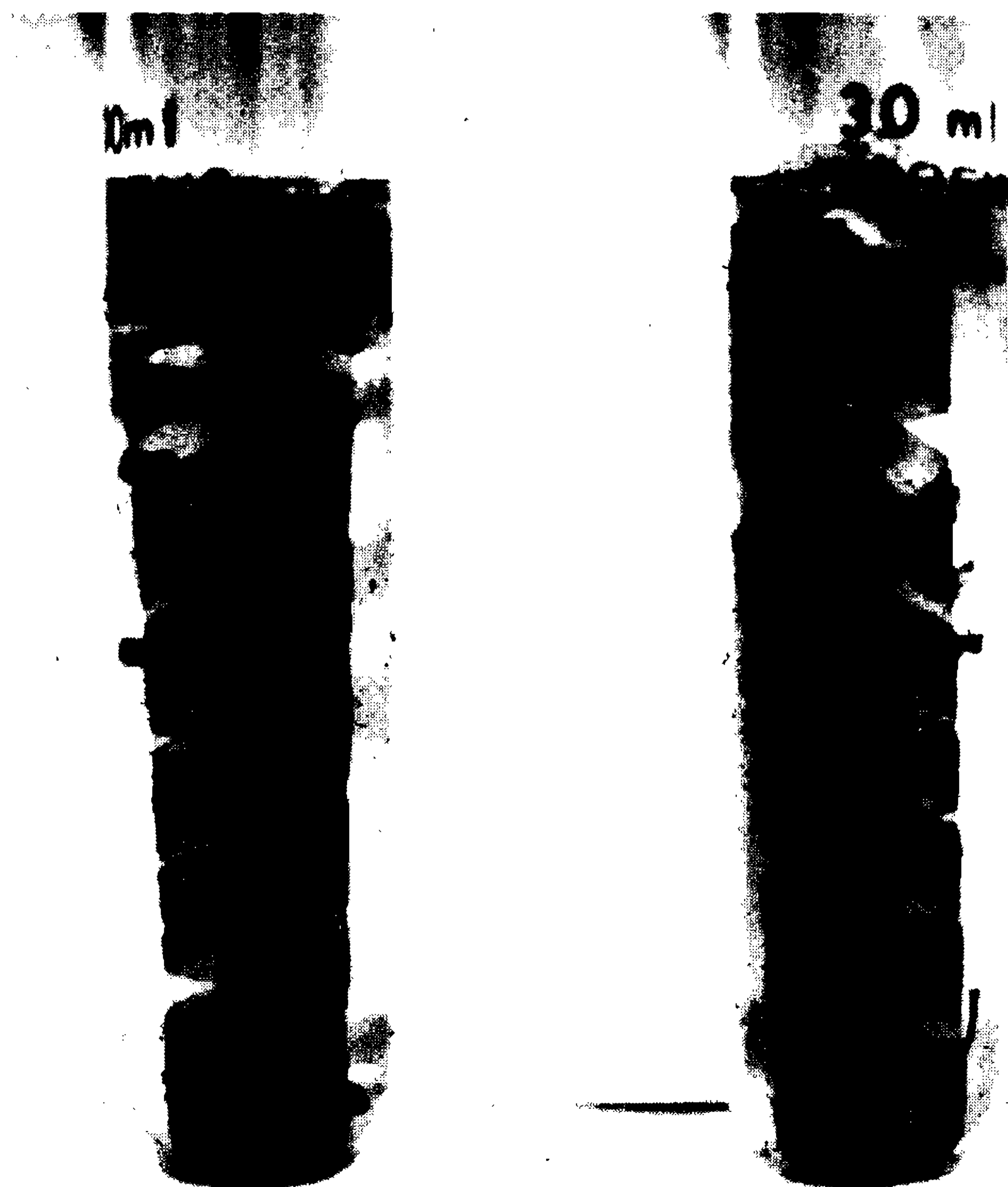


Figure 1. Photograph showing a thick strip of smooth muscle wall of the intestine wrapped on glass rods and stained by the Giemsa's method, after incubation with peripheral lymphocytes of control rabbit and of a Chagas' rabbit.

Effect of antibodies on CNS neurons and on parasympathetic ganglion cells: The effect of humoral factors against CNS neurons and parasympathetic ganglion cells was tested by the incubation with fresh sera from rabbits 4,5 and 6 with high titers (1:256 to 1:512) of anti-*T. cruzi* antibodies. In these experiments culture medium containing 2 to 10 per cent homologous sera were used. In some experiments 10 per cent heterologous serum was used to feed the cultures.

Effect of soluble extracts of T. cruzi on CNS neurons and on parasympathetic ganglion cells: Cultures of CNS neurons and of parasympathetic ganglion cells were maintained in the presence of 100 to 300 µg/ml of a soluble extract of *T. cruzi*, or cytosol. The cultures containing cytosol and also the cultures without cytosol were incubated at 37°C during 2 to 7 days and monitored with the aid of an inverted microscope for the presence of cytopathogenic effect.

Effect of lymphocyte on parasympathetic ganglion and CNS cell cultures. The parasympathetic ganglia isolated from the intestine of normal rabbits were used in these experiments. The myenteric ganglia were incubated with 3×10^6 peripheral blood lymphocytes collected from normal, control rabbits and from rabbits with chronic Chagas' disease, for a period of 18 h at 37°C. The culture medium used contained 5% homologous serum and antibiotics. After incubation the tissues were fixed with methanol and stained by the Giemsa method (Fig. 1). The strip was taken from the glass rods, cut in small segments 5 cm in length and mounted in glass slides for microscopic examination.

In parallel experiments, fetal rabbit CNS cells were seeded in 75 ml culture flasks, as described before. After seven days of cultivation, the CNS monolayers were incubated with 3×10^6 peripheral blood lymphocytes from rabbits with Chagas' disease and from normal, control rabbits. The assesment of cytopathogenic effect was monitored with the aid of an inverted microscope.

Scoring the effect of lymphocytes on ganglia cells. A double-blind method was used to scoring the effect of the lymphocytes on the parasympathetic ganglion cells and on the CNS monolayers. The glass-slides with the segments of the external muscle layer of the intestine containing the parasympathetic ganglia were labelled and given to two observers who did not know the nature of the experiments. The results were scored as follows: 3+ high degree of lymphocyte adherence and neuronolysis; 1+, mild degree of adherence and neuronolysis; 2+ intermediate values; ± random lymphocyte adherence and no cytolysis.

RESULTS

Humoral antibodies and cell-mediated immunity in rabbits with chronic Chagas' disease:

Profiles of the immune responses in six rabbits with chronic *T. cruzi* infection and in two uninfected controls are given in Table I. Their serum antibody levels detected by the indirect hemagglutination tests showed titers that varied from 1:2048 to 1:256. The direct agglutination of trypsinized epimastigote forms of the parasite by anti-*T. cruzi* antisera showed high antibody titers (1:2048 to 1:1024), which did not fall after treatment of the antisera with the reducing agent 2-mercaptoethanol. This observation indicates that antibodies reacting against the parasite were mainly IgG. Both control rabbits gave negative results.

TABLE I
Humoral and Cell-Mediated Immunity in Rabbits with Chronic Chagas' disease

Rabbits	Humoral immunity*			CMI: % Inhibition of cell migration**		
	DA	DA-2ME	IH	TH	MI	CY
Chronic Chagas' disease						
1	—	—	1:1024	43.3±2.9	69.7±2.3	35.7±3.1
2	—	—	1:1024	46.2±4.2	60.3±3.7	40.6±2.9
3	—	—	1:2048	41.3±8.9	60.6±2.9	37.1±1.0
4	1:2048	1:2048	1:256	24.0±9.1	34.8±6.4	30.4±3.0
5	1:1042	1:1024	1:512	61.7±12.1	50.0±9.0	28.9±3.5
6	1:1024	1:512	1:512	31.0±9.3	27.2±2.0	10.1±3.4
\bar{X}				41.2±13.0 ^a	50.4±16.4 ^c	25.4±14.3 ^e
Controls						
7	Neg	Neg	Neg	14.2±1.4	17.3±1.9	20.6±3.2
8	1:16	Neg	Neg	11.2±2.6	0.9±2.8	2.9±1.5
\bar{X}				12.7±2.1 ^b	9.1±11.5 ^d	12.5±11.8 ^f

*DA, direct agglutination of epimastigotes of *T. cruzi* by rabbit antisera; AD-2ME, direct agglutination after treatment of sera with 2-mercaptoethanol. IH, indirect hemagglutination.

**Per cent migration = 100 - (average diameter of mononuclear cell migration exposed to antigen)/(average diameter of mononuclear cell migration in the absence of antigen) x 100. The results were recorded after 18 h of incubation at 37°C. TH, total homogenate of *T. cruzi* forms; MI, microsomal antigen derived from TH; CY, cytosol, soluble supernate of TH.

a vs b, P > 0.01; c vs d, P > 0.005; e vs f, P > 0.2.

The results of assays of inhibition of migration of blood mononuclear cells by *T. cruzi* antigens are shown in Table I. High degrees of inhibition of migration were observed in rabbits with chronic Chagas' disease. The means of inhibition reached 41.2 ± 13.0 per cent when the total homogenate of *T. cruzi* (TH) was used, and reached 50.4 ± 16.4 per cent when the cells were allowed to migrate in the presence of the microsomal antigen (MI) of *T. cruzi*. In contrast, when the blood mononuclear cells from control rabbits migrated in the presence of these antigens the means of inhibition reached 12.7 ± 2.1 per cent (TH) and 9.1 ± 11.5 (MI). These results are statistically significant. However, the degrees of inhibition of migration of the cells from Chagas' rabbits in the presence of cytosol (CY) antigen were not different from those obtained in the control group.

Growth of T. cruzi in CNS cells in the presence of anti-T. cruzi antibodies.

Five day-old cultures of CNS tissue containing a monolayer of glial cells and neurons were infected with 5 x 10⁵ trypomastigotes of *T. cruzi*. The parasites infected the glial cells and multiplied luxuriously in their cytoplasm. The neurons were spared. Further, the glial cells became heavily parasitized after ten days in culture and through cyclical reinfections. After 21 days in culture many glial cells were destroyed by the intracellular parasites, whereas the neurons did not show demonstrable parasites encysted in their cytoplasm. Similar features were observed when the Ernestina strain or the Albuquerque strain of *T. cruzi* were used (Table II).

In other experiments, cultures of CNS tissue were maintained in the presence of anti-*T. cruzi* antisera from controls and Chagas' rabbits (see Table I), containing high titers of specific antibodies. The growth of the CNS cells was not changed by the presence of fresh rabbit anti-*T. cruzi* antisera at 10% concentration in culture medium (v/v). However, CNS cells could not grow in the presence of heterologous serum at 10% concentration. Furthermore, the cultures grown in the presence of anti-*T. cruzi* antibodies could be easily infected with trypomastigote forms of the parasite. The glial cells became heavily infected and no demonstrable cytopathogenic effect was observed in the neurons (Fig. 2).

TABLE II

Growth of *T. cruzi* in CNS cells in the presence of anti-*T. cruzi* antibodies

Rabbit sera*	Strain of <i>T. cruzi</i>	Intracellular growth	
		Glial cells	Neurons &
Chronic Chagas' disease	Albuquerque	+	-
	Ernestina	+	-
Controls	Albuquerque	+	-
	Ernestina	+	-

* 10% sera from rabbits 1 to 8 (see Table I) in MEM were used to feed the monolayers.

& The neurons were not parasitized by *T. cruzi* and no cytopathogenic effect could be observed that would indicate the release of a toxin by the parasite encysted in the glial cells.

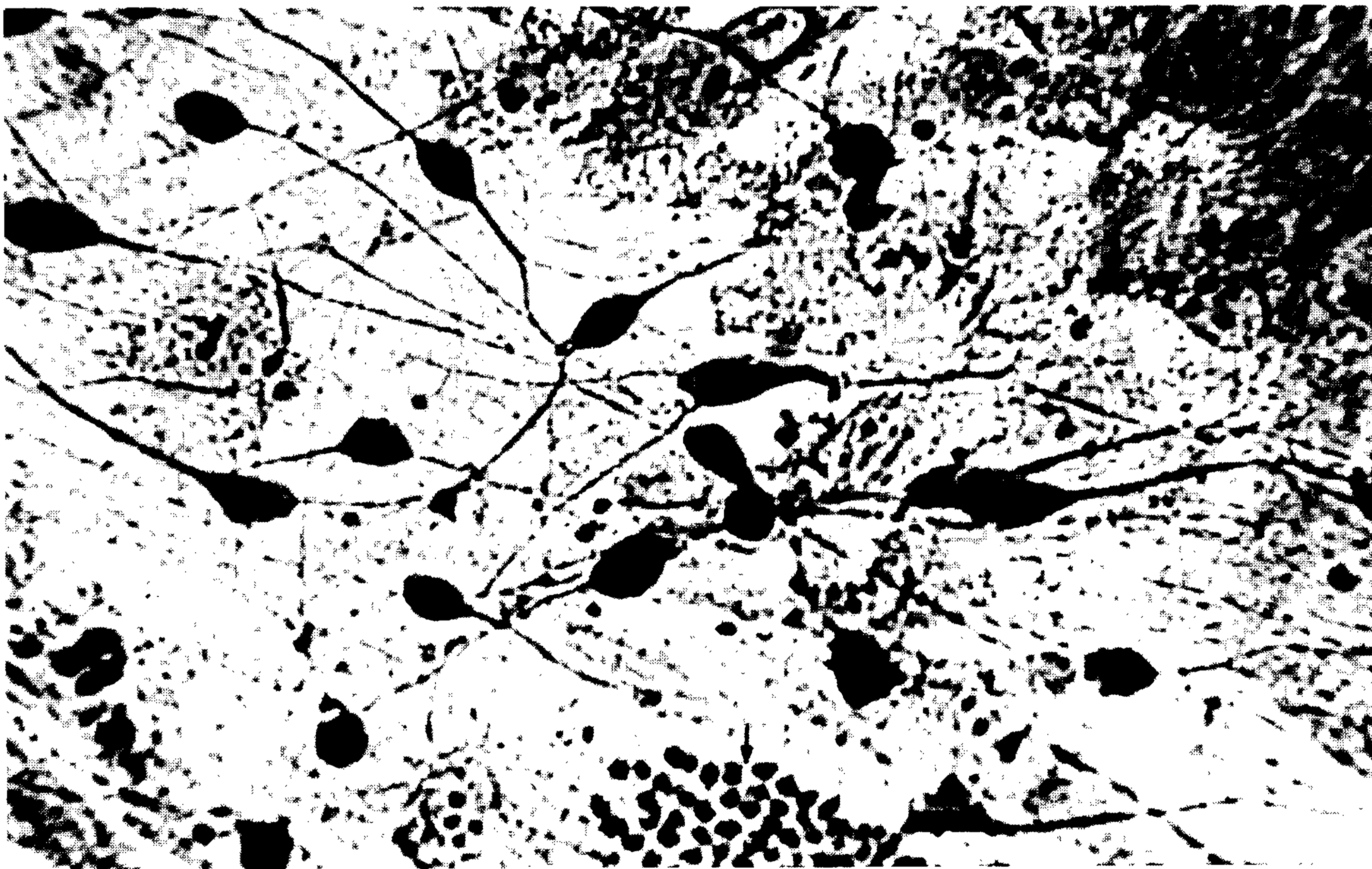


Figure 2. Microphotograph of CNS cell culture grown in the presence of anti-*T. cruzi* antisera and infected with *T. cruzi*. Note the CNS neurons on top of glial cells and absence of cytopathogenic effect. The arrow shows a glial cell heavily parasitized.

Growth of rabbit CNS neurons and of myenteric ganglion cells in the presence of soluble extracts of T. cruzi forms and of anti-T. cruzi antibodies.

The CNS cell cultures grown in MEM with 5% homologous serum received 100 to 300 $\mu\text{g/ml}$ of a soluble extract of *T. cruzi* (see material and methods). These cultures were incubated at 37°C and allowed to grow for seven days in the presence of this *T. cruzi* extract. Every time the culture medium was removed an equal amount of the *T. cruzi* extract was added to the culture medium. At the end of seven days the glial cells had not stopped growing and no demonstrable toxic effect was observed on the neurons. How-

ever, the CNS cell cultures could not be maintained alive when the quantity of the homologous serum was reduced to the concentration of 2% in the culture medium in spite of the presence of 300 $\mu\text{g/ml}$ of the *T. cruzi* extract (Table III). Further, CNS cell cultures were grown in the presence of fresh rabbit anti-*T. cruzi* antisera plus 100 to 300 $\mu\text{g/ml}$ of soluble extract of the parasite, and no demonstrable cytopathogenic effect could be observed on the neurons.

TABLE III

Growth of CNS neurons and of myenteric ganglion cells in the presence of soluble extracts of *T. cruzi* forms and of anti-*T. cruzi* antibodies*

<i>Addition to cultures</i>	<i>CNS Neurons</i> &	<i>Parasympathetic neurons</i> §
100 μg of cytosol/ml	G	G
300 μg of cytosol/ml	G	G
300 μg of cytosol/ml +		
5% Chagas' rabbit sera	G	G
300 μg of cytosol/ml +		
2% Chagas' rabbit sera	D	ND

* Cultures of rabbit fetal CNS tissue and of myenteric ganglia cells were established and maintained in MEM containing 5% homologous sera.

§ The cultures of CNS neurons were maintained for 7 days and the parasympathetic ganglia were maintained for 2 days, both in the presence of *T. cruzi* extracts (cytosol) and of anti-*T. cruzi* antisera.

G, growth; D, death; ND, not done.

In other experiments, cultures of parasympathetic ganglion cells maintained in MEM with 5% homologous serum were incubated with 100 to 300 μg of the *T. cruzi* soluble extract. After 48 h at 37°C, the strip of intestinal smooth muscle containing the parasympathetic ganglion cells were fixed in methanol, stained by the Giemsa's method and mounted in glass slides. The histologic features observed in the myenteric ganglia were similar to those found in the preparations cultivated without *T. cruzi* soluble extract. No demonstrable toxic effect was observed in the neuronal cells. The addition of fresh anti-*T. cruzi* antisera did not result in any demonstrable change.

Effect of immune lymphocytes from rabbits with chronic Chagas' disease on myenteric, parasympathetic ganglion cells.

In these experiments blood lymphocytes collected from normal, control rabbits and from rabbits with chronic Chagas' disease (Table IV) were incubated for 18h at 37°C, with parasympathetic ganglion cells. When 3×10^6 immune lymphocytes collected from rabbits 1 to 6 with chronic Chagas' disease were incubated with parasympathetic ganglion cells, penetration and adherence of *T. cruzi*-immune lymphocytes on the surface of the neurons was observed. In some experiments, incrustation of immune lymphocytes

TABLE IV

Effect of immune lymphocytes from rabbits with chronic Chagas' disease on myenteric, parasympathetic ganglion cells

<i>Rabbits</i>	<i>Lymphocyte adherence and neuronolysis*</i>
Chronic Chagas' disease	
1	+++
2	++
3	+++
4	++
5	++
6	+
Controls	
7	±
8	±

* 3+, high degree of lymphocyte adherence and neuronolysis; 1+, mild degree of adherence and neuronolysis; 2+, intermediate values; ±, random lymphocyte adherence and no neuronolysis.



Figure 3. Microphotograph of a strip of intestinal smooth muscle incubated with 3×10^6 immune lymphocytes of a rabbit with chronic Chagas' disease. Note penetration and adherence of immune lymphocytes to the parasympathetic ganglion cells (arrows).

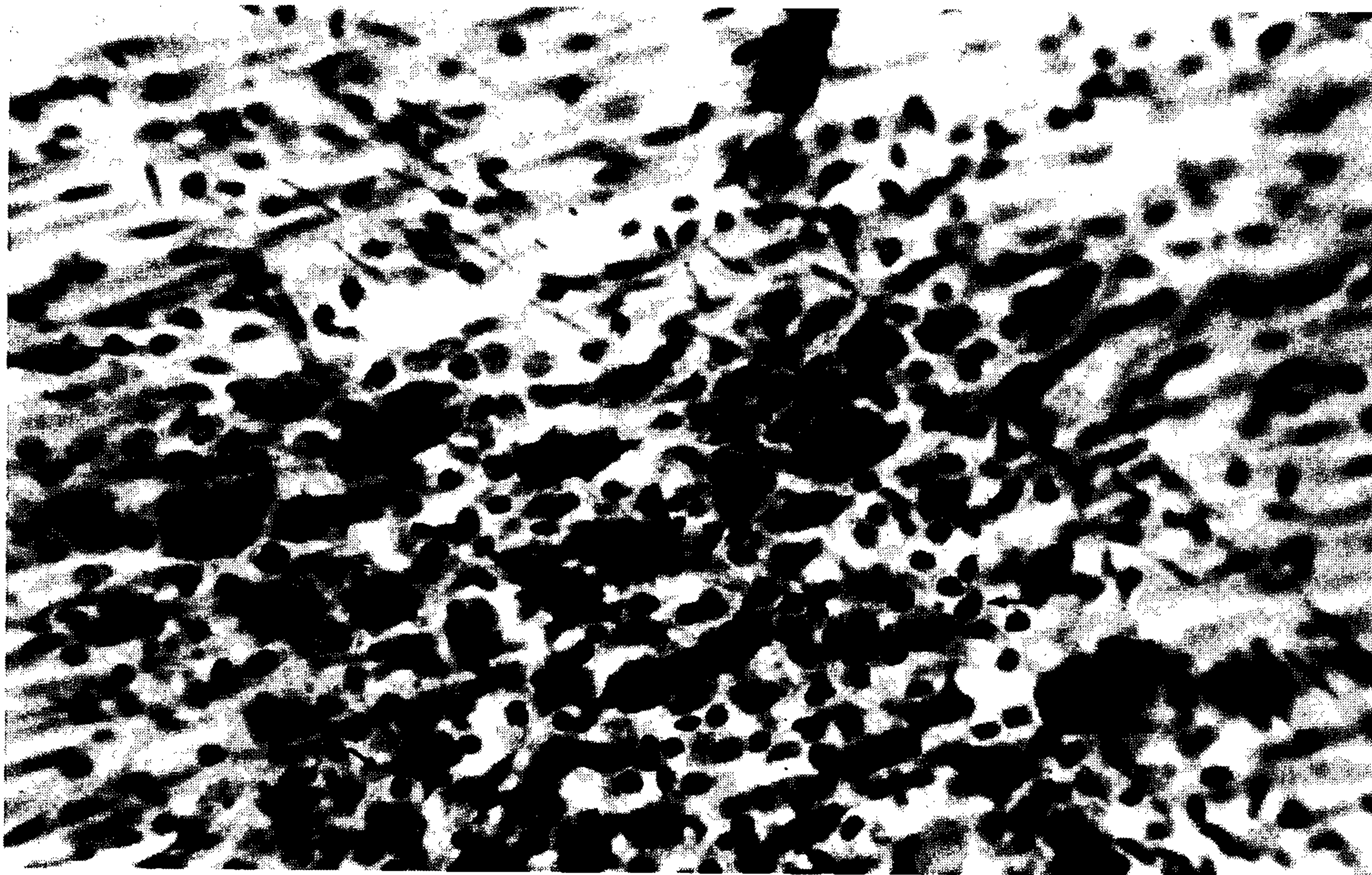


Figure 4. This picture shows the appearance of parasympathetic ganglion cells after 18 h incubation, at 37°C, with *T. cruzi*-sensitized lymphocytes.

on the cytoplasm of the neurons was suggestive of neuronolysis. Several degrees of immune lymphocyte adherence and neuronolysis were observed when the cells were obtained from rabbits with chronic Chagas' disease, and a 1+ to 3+ were scored. (Figs. 3 and 4). In control experiments, non-immune cells from control rabbits showed random lymphocytic adherence and no neuronolysis, and a \pm was scored (Table IV).

Of great interest, incubation of 3×10^6 immune lymphocytes with CNS cell cultures did not show any of those features described for the parasympathetic ganglia cells. The *T. cruzi*-immune lymphocytes did not adhere to the surface of CNS neurons and no cytopathogenic effect could be demonstrated. The nervous cells were not destroyed by the immune lymphocytes, after 18 h of incubation at 37°C. This observation was similar to that obtained when normal, non-immune lymphocytes were incubated with CNS cells.

DISCUSSION

In 1955, Köberle & Nador showed that the mega conditions seen in Chagas' disease are related to depopulation of parasympathetic, myenteric ganglion cells of the digestive tube. The ganglion cell depopulation has been demonstrated in cases of megaesophagus and/or megacolon and, also, in Chagas' patients who died with the cardiac disease. It is of considerable interest to notice that Chagas' disease may show various degrees of ganglion cell depopulation even if cardiac lesions are not present (Andrade & Andrade, 1967). Similar features have been observed in animals (Teixeira, Teixeira & Santos-Buch, 1975; Tafuri et al., 1979) naturally or experimentally infected with *T. cruzi*. Many hypotheses have been raised as attempts to explaining the mechanisms whereby ganglion cells are destroyed. The aim of this work was to examine possible mechanisms of parasympathetic ganglion cell destruction in Chagas' disease.

Chagas' megaesophagus and megacolon do not seem to be directly related to the presence of the parasite. Usually, these manifestations are found in patients with chronic Chagas' disease, when *T. cruzi* is not found encysted in the tissue cells. *T. cruzi* can parasitize any cell type, but the finding of the parasite in parasympathetic ganglion cells is very rare (Köberle & Alcântara, 1960). The occasional finding of parasitism of ganglia cells was described in mice overwhelmingly infected (Alcântara & Oliveira, 1964; Köberle, 1957) and treated with immunosuppressive drug (Andrade & Andrade, 1966), which did not show mega condition. Despite these evidences against a direct action of the parasite on the ganglion cells, we examined the effect of *T. cruzi* against CNS neurons *in vitro*. The infection of CNS cell cultures with trypomastigotes of two strains of *T. cruzi* resulted in parasitism of the glial cells, whereas the neurons were spared. This observation is in keeping with many authors (Vianna, 1914; Crowell, 1923; Teixeira, Roters & Mott, 1970) who have described a marked tropism of the parasite for the glial cells, in contrast to the unusual finding of parasitism of neuronal cells, in the acute phase of experimental Chagas' disease. However, Meyer & Machado (1979) showed that chick embryo spinal ganglia cells can be parasitized by *T. cruzi in vitro*. We believe that the different results described here can be explained on the basis of the stage of differentiation and the source of the nervous cells used. In view of the evidence presented in this paper it is unlikely that the parasympathetic ganglion cells are directly destroyed by the parasite. In favor of this observation is the manifestation of the mega disease in the chronic phase of Chagas' disease, when the parasite is not found in the lesion.

Furthermore, the experiments described here allowed us to examine the possibility that *T. cruzi* can secrete a neurotoxin *in vitro*. After 21 days of infection the glial cells were heavily parasitized but the neurons did not show any evidence of cytopathogenic effect that could be attributed to a neurotoxin. In other experiments, cultures of CNS cells and of parasympathetic ganglion cells were maintained alive in the presence of 100 to 300 $\mu\text{g/ml}$ of a fresh, soluble extract of *T. cruzi*. The histologic features observed in these cells, after staining by the Giemsa's method, did not show any cytopathogenic effect. Therefore, this paper confirms previous data by Mussachio & Meyer (1959) who did not observe any evidence for a neurotoxin in Chagas' disease.

The development of the megas in late stages of chronic Chagas' disease, in the absence of the parasite encysted in tissue cells, has indicated the necessity of assessing what a role immune mechanisms might play in nervous cell destruction in this disease. In these studies, CNS cells and parasympathetic ganglion cells were cultivated in the presence of fresh rabbit anti-*T. cruzi* antisera with high titers of specific antibodies. These experiments showed absence of demonstrable cytopathogenic effect. Further, the effect of immune-complexes was studied by the addition of soluble *T. cruzi* antigens to the cultures containing anti-*T. cruzi* antibodies. No demonstrable cytopathogenic effect was observed on the neurons. Therefore, these experiments failed to demonstrate any role of antibodies and immune-complexes in the pathogenesis of parasympathetic neuronal cell destruction, which is consistently described in Chagas' disease.

However, many authors (Tafari et al., 1979; Ribeiro dos Santos, 1977; Teixeira, 1977) have reported on a role played by immune mechanisms on the pathogenesis of myenteric ganglia cell destruction, in Chagas' disease. Tafari et al. (1979) showed that mice infected with 5,000 blood trypomastigotes of the Y strain of *T. cruzi* survived the acute phase of infection. When the animals were sacrificed in the chronic phase of infection, the lesions found in parasympathetic ganglia were not related to parasitism of neurons or glial cells. In fact, it was shown by light and electronic microscopy that the lymphocytes seem to attack the ganglion cells. This lesion appeared to be related to lymphocyte adherence and penetration in the cytoplasm of the ganglion cells, which displayed morphologic signs of desintegration.

In the experiments reported here we have described a selective affinity of *T. cruzi*-immune lymphocytes to the myenteric, parasympathetic ganglion cells. The immune

lymphocytes used in these studies were collected from rabbits with chronic Chagas' infection, whose blood mononuclear cells had shown significant inhibition of migration in the presence of *T. cruzi* antigens. The incubation of 3×10^6 immune lymphocytes from these infected animals with cultures of intestinal smooth muscle layer containing parasympathetic ganglia, resulted in penetration and adherence of the lymphocytes to the ganglion cells. In many experiments, incrustation of immune lymphocytes on the cytoplasm of neurons was a good indication of neuronolysis. None of these features were observed when non-immune lymphocytes from control, normal rabbits were used.

The degree of specificity of this reaction was indicated by other experiments in which the affinity of *T. cruzi* immune lymphocytes to CNS neurons was tested. In these experiments cultures of cortical CNS tissue were incubated with immune lymphocytes of chronic Chagas' rabbits. After 18 h of incubation at 37°C the glial cells and CNS neurons were not attacked by lymphocytes, which remained in the supernatant nutrient medium. These results parallel those obtained when cultures of cortical CNS tissue were incubated with non-immune lymphocytes from control rabbits. Therefore, the selective affinity and lysis of parasympathetic ganglia cells by *T. cruzi*-immune lymphocytes seem to be a unique example of the high degree of specificity of autoimmunity in Chagas' disease. Yet, the molecular basis of this specificity remains to be elucidated. We postulate that the antigenic determinant that leads to cross-reaction of *T. cruzi*-immune lymphocytes to parasympathetic neurons might be related to a product of neurosecretion that is not present in CNS neurons. In favor of this postulate is the demonstration of lymphocytic infiltrates and parasympathetic ganglion cells destruction in rabbits and patients with chronic Chagas' infection, in the absence of encephalitis (Teixeira, 1977; Queiroz, 1978). Further, this postulate bears some similarity to what has been described in experimental autoimmune myasthenia gravis. This disease is related to an immune response to nicotinic acetylcholine receptors, which gives rise to a destructive cellular attack directed at the postsynaptic membrane of muscle (Lennon et al., 1977).

Santos-Buch & Teixeira (1974) and Teixeira et al. (1975) have shown that delayed-type hypersensitivity plays a major role in the pathogenesis of the myocarditis of Chagas' disease. A cross-reactive microsomal antigen of heart cell was shown which inhibited the migration of blood mononuclear cells from Chagas' rabbits. The recognition of cross-reactive host cell antigen by *T. cruzi*-immune lymphocyte was suggested as the pathogenic basis of tissue injury in Chagas' disease. This demonstration of selective affinity and cytotoxicity of *T. cruzi*-immune lymphocytes to myenteric, parasympathetic ganglion cells suggest that delayed hypersensitivity is a basis mechanism related to the pathogenesis of the mega conditions of Chagas' disease. However, the data presented here also suggest that the immune lymphocytes responsible for ganglion cell lysis might be different from those involved in heart cell destruction. This would explain the occurrence of mega disease even if carditis is not present. These data indicate that at least two clones of cytotoxic lymphocytes are involved in autoimmunity in Chagas' disease.

RESUMO

O megaeosôfago e o megacolon endêmicos na América do Sul estão relacionados à doença de Chagas. Estas condições clínicas são encontradas em pacientes com infecção chagásica crônica, quando o parasito não é demonstrado nas lesões caracterizadas por despovoamento de células neuronais parassimpáticas. Nos experimentos descritos aqui nós demonstramos uma afinidade seletiva de linfócitos imunes, sensibilizados pelo *T. cruzi*, para neurônios de gânglios mioentéricos. Os linfócitos imunes citotóxicos aderem nas células ganglionares, produzindo neuronólise. Isto não se observa quando linfócitos não-imunes são usados, ou quando os linfócitos imunes são colocados na presença de neurônios do sistema nervoso central. Esta é uma demonstração do alto grau de especificidade da destruição de neurônios parassimpáticos na doença de Chagas. Postulamos que a rejeição de neurônios parassimpáticos deve estar relacionada ao reconhecimento de um

determinante antigênico de reação cruzada, o qual seria secretado exclusivamente pelos neurônios parassimpáticos. Em favor desta interpretação temos a observação de infiltrados linfocitários com destruição de células ganglionares parassimpáticas em pacientes com infecção chagástica crônica, na ausência de encefalite.

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